Replace the first full paragraph on page 2 (lines 6-7) with the following new paragraph rewritten in clean form:

A2

In general, the invention features a protein having a covalently bonded puromycin-tag, the tag being positioned at the C-terminal end of the protein.

Replace the second full paragraph on page 2 (lines 8-16) with the following new paragraph rewritten in clean form:

A3

In preferred embodiments, the tag is a small molecule (for example, biotin); the tag is a detectable label (for example, fluorescein, rhodamine, or BODIPY, or derivatives thereof); the tag is a functional group (for example, a functional group having a reactivity orthogonal to the reactivity of one of the protein's functional groups); the tag is a tether for attachment to a solid support (for example, a column, bead, or chip); the tag is one member of a specific binding pair; the tag is a phenyl diboronic acid derivative; the puromycin-tag further includes a nucleotide sequence positioned between the tag and the puromycin; and the nucleotide sequence is between about 1-200 nucleotides in length.

Replace the third full paragraph on page 2 (lines 17-22) with the following new paragraph rewritten in clean form:

A4

In a related aspect, the invention features a method for C-terminal protein tagging, involving (a) providing a nucleic acid sequence encoding the protein; (b) translating the sequence under conditions in which translation stalls at the 3' end of the sequence, forming a stalled translation complex; and (c) contacting the stalled translation complex with a puromycin-tag under conditions in which the puromycin-tag is covalently bonded to the C-terminus of the protein.

Replace the paragraph beginning on page 2, line 23 and ending on page 3, line 13 with the following new paragraph rewritten in clean form:

A5

In preferred embodiments, the tag is attached to the 5'-hydroxy group of puromycin; the tag is attached to the 5'-hydroxy group of the puromycin through a phosphate group; the nucleic acid sequence encoding the protein contains no stop codons; the translation step is carried out in the substantial absence of at least one translation release factor; the 3'-end of the nucleic acid sequence encoding the

protein is covalently linked to a DNA oligomer; the tag is a small molecule (for example, biotin); the tag is a detectable label (for example, fluorescein, rhodamine, or BODIPY, or a derivative thereof); the tag is a functional group; the protein has a first functional group and the tag is a second functional group, wherein the first functional group has a reactivity orthogonal to the reactivity of the second functional group; the tag is a tether for attachment to a solid support (for example, a column, bead, or chip); the tag is one member of a specific binding pair; the tag is a phenyl diboronic acid derivative; the puromycin-tag further includes a nucleotide sequence positioned between the tag and the puromycin; and the nucleotide sequence is between about 1-200 nucleotides in length.

A5

Replace the paragraph beginning on page 3, line 17 and ending on page 4, line 1 with the following new paragraph rewritten in clean form:

By a "puromycin-tag" is meant puromycin having a covalently bonded structural or functional moiety which is not native to the puromycin molecule and which is chosen from the group consisting of a detectable label, a chemically reactive functional group, a small molecule, a protein or peptide, a peptoid, a naturally occurring or non-naturally occurring polymer, a solid-phase bound tether, a carbohydrate, or a nucleic acid (preferably, of between about 1-200 nucleotides) which does not encode the protein to which the puromycin-tag is itself covalently linked. By a "nucleic acid" is meant any two or more covalently bonded, naturally occurring or modified nucleotides and includes DNA, RNA, and PNA. Preferred puromycin-nucleic acid tags include 5'-C-C-puromycin-3'.

A6

Replace the second full paragraph on page 8 (lines 16-23) with the following new paragraph rewritten in clean form:

Any appropriate type of ligation chemistry may be exploited to attach the tag to the puromycin moiety and, for example, to the 5'-hydroxy group of the puromycin. In a preferred embodiment of the invention, the puromycin-tags are synthesized using standard solid phase techniques, for example, as outlined in Figure 2. Commercially available phosphoramidites of biotin or fluorescein (Glen Research), for example, may be used to derivatize the 5'-terminus of puromycin or X_n-puromycin. These reactions may be carried out, for example, as described in Oligonucleotide Synthesis: A Practical Approach, ed. Gait, M.J. (IRL, Oxford).

A7

Replace the first partial paragraph on page 9 (lines 1-14) with the following new

partial paragraph rewritten in clean form:

A7

These derivatives include puromycin or X_n-puromycin linked to small molecules, for example, X_n-puromycin-5'-phosphate carrying a tethered biotin derivative (Figure 4); such a puromycin- or X_n-puromycin- tag may be used to attach a C-terminal biotin label to a protein, for example, for affinity purification. In a further example, a puromycin- or X_n-puromycin- tag may act as a bifunctional reagent by using a puromycin or X_n-puromycin derivative which contains both attachment and detection groups, for example, as shown in Figure 5. In this example, an X_n-puromycin derivative is tethered through its 5'-phosphate to both biotin and fluorescein moieties using standard oligonucleotide synthesis techniques. Attachment groups may include, without limitation, biotin, phenyl diboronic acid/salicylhydroxamic acid, 1,2-amino thiol, or ketone. Detection groups may include, without limitation, fluorescein or derivatives thereof, rhodamine or derivatives thereof, or BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene, Molecular Probes, Eugene, OR) or derivatives thereof.

Replace the paragraph beginning on page 9, line 24 and ending on page 10, line 5 with the following new paragraph rewritten in clean form:

A8

The scheme in Figure 3 outlines the synthesis of puromycin- or X_n-puromycin- tags which include a terminal modification that introduces a terminal amino or thiol functionality into the puromycin or X_n-puromycin intermediate. These reactions are again carried out as described above for Figure 2. As illustrated in Figure 7, these reactive moieties are then used to introduce non-protein functional groups, for example, a ketone, into the puromycin- or X_n-puromycin- tag.

Replace the first full paragraph on page 11 (lines 3-8) with the following new paragraph rewritten in clean form:

A9

In yet another embodiment, puromycin may be linked to polymers. In one particular example, puromycin may be attached to an oligonucleotide using previously described methods (Szostak et al., WO 98/31700; Szostak et al., U.S.S.N. 09/247,190 (1999), now U.S. Patent No. 6,261,804 B1; Roberts & Szostak (1997) Proc. Natl. Acad. Sci. USA 94:12297-12302) for the purpose of sequence-specific hybridization to a solid phase.

Replace the second full paragraph on page 11 (lines 9-14) with the following new paragraph rewritten in clean form:

A10

An appropriate puromycin- or X_n-puromycin- tag may also be used for the preparation of protein-protein conjugates. In one example, a puromycin or X_n-puromycin dimer (Figure 10) may be added to stalled ribosome complexes to generate protein homodimers. Puromycin or X_n-puromycin dimers are prepared as described above for Figure 2 using a second puromycin or puromycin derivative as a tag.

Replace the paragraph beginning on page 12, line 14 and ending on page 13, line 4 with the following new paragraph rewritten in clean form:

A11

To maximize the yield of the C-terminally tagged product, the tag is preferably attached to the full-length peptide or protein following translation of the open reading frame. This can be achieved by stalling the ribosome as an mRNA-ribosome-peptidyl complex after translation of the coding sequence. Ribosome stalling at the 3'-end of the open reading frame may be accomplished by any of a number of different methods. In one preferred approach, the message is engineered to be devoid of stop codons. As a result, release factors cannot bind, and the ribosome stalls (see, for example, Hanes & Plueckthun (1997) Proc. Natl. Acad. Sci. USA 94: 4937-4942). In another preferred approach, a DNA oligomer may be linked to the end of the message causing the ribosome to pause; this technique is described in Szostak et al., WO 98/31700; Szostak et al., U.S.S.N. 09/247,190 (1999), now U.S. Patent No. 6,261,804 B1; and Roberts & Szostak (1997) Proc. Natl. Acad. Sci. USA 94: 12297-12302). Alternatively, an in vitro translation lysate may be utilized which is devoid of release factors, as described in Lipovsek et al., Methods for Optimizing Cellular RNA-Protein Fusion Formation, U.S.S.N. 60/096,818; U.S.S.N. 09/374,962 (1999), now U.S. Patent No. 6,312,927; and WO 00/09737.

In the claims:

Amend claims 14, 15, 18, 20, 22, 24-26, and 28-30 as follows.

A12

14. (Amended) A method for C-terminal protein tagging, comprising

(a) providing a nucleic acid sequence encoding a protein;